THE INFLUENCE OF OXYGEN TENSION UPON THE RESPIRATION OF UNICELLULAR ORGANISMS.

WILLIAM R. AMBERSON.

(From the Department of Physiology, School of Medicine, University of Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Mass.)

Our knowledge of the influence of oxygen tension upon the oxygen consumption of unicellular organisms is quite incomplete. The literature contains many studies of the influence of oxygen tension changes upon growth and activity of such forms, but relatively few direct measurements of oxygen consumption have been made. In some studies in which the consumption has been measured the problem has been complicated by changes in the number of respiring cells during the course of the experiment. This would appear to be true of such observations as those of Stephenson and Whetham (1924) who have found that the oxygen intake of *B. coli* is much greater in pure oxygen than in air, and of Novy and Soule (1925) who report that the tubercle bacillus grows best in an atmosphere containing 40–50 per cent. oxygen, the growth and the oxygen consumption falling off progressively above and below this value. It is not possible to infer that a change in division rate indicates a change in the oxygen intake of the individual bacterium. The influence of the oxygen tension may be more indirect, possibly through the formation of such growth-promoting substances as Burrows (1924) has described, whose production is increased by an increased oxygen supply.

In other studies of bacterial respiration in which there has probably been no significant change in the number of respiring cells, Pütter (1924) and E. N. Harvey (1926) have secured evidence that the respiratory rate is not influenced by changes in the oxygen tension. In unicellular animal organisms the weight of the somewhat meagre evidence so far secured indicates that oxygen consumption is independent of oxygen tension over a wide range. Lund (1918) found this to be true for *Paramecium*. Henze (1910) and Warburg (1908) found a similar situation in

sea-urchin eggs, in which there was little change in oxygen intake when the oxygen tension varied from double that in air to one-fourth of the same value.

In all of the studies in this last group in which oxygen has actually been measured, the Winkler method has been employed. It is well known that this method, while very satisfactory for the determination of dissolved oxygen in pure water or in salt solutions, becomes untrustworthy when organic material is present in the fluids tested. Heilbrunn (1915) and others have objected to the use of the method in the study of heavy suspensions of protozoa and marine eggs. The presence of iron, found by Warburg (1914) to be contained in sea-urchin eggs in considerable amounts, is known to introduce large errors in the titration. (See Alsterberg, 1926.)

I became interested in this problem after making the observation (1924) that the oxygen consumption of a number of marine invertebrates is directly proportional to the oxygen tension in the sea water, over a considerable part of the normal physiological range. This observation has led me to a reëxamination of the problem in other forms. The present communication deals with some results obtained on unicellular materials in an attempt to confirm the conclusions of previous workers by methods not open to the criticisms which can be leveled against the Winkler technique. This confirmation has been secured. The data are submitted in support of the older observations, and as giving a more complete account of the oxygen tension relationships in the *Arbacia* egg than has previously been published.

On the technical side an attempt has been made to apply standard methods of gas analysis to the study of the problem. Novy and his collaborators have previously successfully used such methods in their study of bacterial respiration. I find that the oxygen consumption of unicellular animal organisms can be similarly followed by such methods, with an accuracy at least as good as that possible in human and mammalian metabolic studies. The carbon dioxide production is more difficult to determine because of the high solubility of the gas in the liquid phase, and the possibility of its chemical fixation. No great reliance can therefore be placed upon the carbon dioxide values given below, or upon the

respiratory quotients calculated. The large variations in the value of the quotient is sufficient to indicate the magnitude of the errors which must be present in the determination of carbon dioxide. My main concern has been to study the oxygen consumption.

Experiments with *Paramecium*.

A group of experiments was first carried out with *Paramecium*, in an attempt to develop a satisfactory technique. For several reasons the data obtained are not as complete or accurate as the values secured later on *Arbacia* eggs. The results are, however, fairly consistent and give a satisfactory confirmation of Lund's report on this organism.

A thick suspension of the protozoa was prepared by centrifuging several liters of fluid from a number of cultures. The organisms were then washed through several changes of tap water, being concentrated with the centrifuge after each washing. The suspension in its final form was practically free from bacteria. The cultures were never entirely pure, but *P. caudatum* always constituted at least 95 per cent. of the protozoa present. The presence of other unicellular organisms, either animal or plant, cannot appreciably have modified the results.

A preliminary obstacle was encountered when it was observed that it is exceedingly difficult to secure two samples of such a suspension which will contain the same number of animals. This difficulty arises from the high mobility of the organisms which are negatively geotropic, and tend to rise to the surface even while the sample is being drawn. After many unsuccessful attempts to secure two identical samples, the procedure was abandoned. In its stead it was found possible to carry out two consecutive measurements of respiration upon the same suspension, the first at atmospheric pressure, the second at some lower or higher pressure. Under the conditions of the experiments division was absent, yet the measurements were completed before starvation intervened.

20 cc. of the suspension finally obtained were introduced into a cylindrical glass vessel, of about the size and form of a Haldane gas collecting tube. This tube was fitted with three-way stopcocks at both ends. The volume was 80.85 cc. After the introduction of the suspension the volume of gas in the tube was, therefore,

60.85 cc. Air delivered by a pump under a small pressure was now bubbled through the suspension for five minutes. This air was taken by the pump from a large room in the basement of the medical building; its oxygen content was slightly lower, and its carbon dioxide content slightly higher, than in outside air. The actual percentages were determined by later analysis. At the end of the equilibration period the tube, completely filled with the room air, and with the suspension, in gaseous equilibrium with this air, was closed off, leaving the contained gas completely saturated with water, at atmospheric pressure, and at approximately 25° C., the temperature of the room. The tube was then placed horizontally within a water bath at a temperature of 25° C. ± .2°. From time to time the tube was gently rocked by hand to keep the suspension approximately in gaseous equilibrium with the air above it. At the end of three hours the tube was removed and the suspension vigorously shaken into complete equilibrium with the gaseous phase. A sample of the contained gas was now withdrawn into a Bailey collector, and set aside for later analysis.

As quickly as possible the same suspension was again equilibrated with room air. The tube was then partially exhausted by a water pump, the residual pressure being measured by a mercury manometer connected with one inlet. Upon the attainment of the desired low pressure the stopcocks were closed, and the tube placed again within the water bath. At the conclusion of a second three hour period the gas in the tube was brought to atmospheric pressure and a sample collected. At the end of this second period the organisms were alive and active.

The gas samples were now analyzed by the use of a Haldane-Henderson gas analyser. Whenever possible duplicate or triplicate determinations were made, and the results averaged. Assuming the gaseous solubilities to be those given by the standard tables for pure water at this temperature, the total oxygen and carbon dioxide present at the beginning and at the end, in both air and water, were now calculated, the usual corrections for barometer, water vapor, etc., being applied.

The results obtained in fourteen experiments are given in Table I. It is seen that the oxygen intake is practically constant from 200 to 50 mm. Hg partial pressure of oxygen. Below 50 mm. the

values are somewhat reduced, but down to 11 mm. the intake is still at least 80 per cent. of that at atmospheric pressure. Since, in these experiments, an oxygen gradient must have been present from air to water, the actual tensions in the water were somewhat lower than those given in the table, which represent the tensions in the air. The ability of these organisms to utilize oxygen at low tensions therefore becomes even more evident.

TABLE I.

RESPIRATION OF *Paramecium* AT DIFFERENT OXYGEN TENSIONS.

Experiment.	Oxygen Pressure in Second Period.	Respiration in First Period.			Respiration in Second Period.			Ratio between O_2 Consumption in Second Period and that in First Period.
		O_2 Cons.	CO_2 Prod.	R. Q.	O_2 Cons.	CO_2 Prod.	R. Q.	
	mm. Hg.	*c.c.	c.c.		c.c.	c.c.		
1	208–192	1.030	.703	.683	1.027	.753	.733	.997
2	211–195	1.107	.663	.598	1.167	.762	.653	1.054
3	154–139	.969	.565	.583	1.025	.640	.625	1.058
4	154–135	1.345	.849	.632	1.390	.903	.649	1.033
5	122–109	.933	.763	.817	1.029	1.016	.986	1.103
6	92–68	2.088	1.490	.714	2.002	1.446	.722	.952
7	91–74	1.216	1.086	.893	1.245	1.204	.967	1.024
8	70–48	1.654	1.302	.787	1.612	1.448	.898	.975
9	70–60	.698	.390	.559	.724	.458	.633	1.037
10	70–57	1.131	.676	.598	.973	.553	.568	.860
11	60–42	1.645	1.028	.686	1.440	1.008	.699	.875
12	49–28	1.592	1.093	.686	1.546	1.115	.721	.971
13	28–11	1.146	.766	.668	.977	.638	.652	.853
14	28–11	1.642	1.134	.691	1.290	1.038	.804	.786
		Average R. Q.		.685			.736	

* Volume measured at 760 mm. Hg and 0° C.

The average of the respiratory quotients obtained in twenty-eight determinations comes out to be .710. Considering the wide range of the individual values it is hardly possible to attach any great significance to this figure, although it may be taken to suggest the presence of a fat metabolism under the conditions of the experiment, when the normal food supply is absent.

These preliminary experiments indicated that the method is applicable to such problems, but certain difficulties were encountered which made it advisable to complete the study on another material.

These consisted in (1) the impossibility of controlling the activity of the organisms, (2) the manipulation of gases at pressures very much below atmospheric, which prevented the exploration of very low oxygen tensions, and (3) the lack of complete gaseous equilibrium between air and water during the course of the experiment. The study was therefore continued with a modified method at Woods Hole on fertilized *Arbacia* eggs, which have no independent motility during the first hours of their development.

Experiments with Fertilized *Arbacia* Eggs.

In these experiments it has been found possible to secure two suspensions of eggs containing equal numbers of cells, whose respiratory exchanges check well with each other when the two are studied simultaneously under identical conditions. The eggs were freed from ovarian debris and body fluid and washed through several changes of sea water. A heavy suspension of cells was secured by permitting the eggs to sediment in a large beaker and then pouring off the greater part of the supernatant sea water. These were then fertilized. About ten minutes after fertilization two 60 cc. samples of this suspension were taken up by pipette and introduced into two tubes similar to that used for *Paramecium* but of a somewhat larger volume.

The lower oxygen tensions were secured by mixing oxygen and nitrogen, or air and nitrogen, in the desired proportions. Eight liters of such a gaseous mixture were collected in a large bottle, over water. One tube (*B*) was then brought into equilibrium with this mixture, the gas being bubbled through the suspension for at least five minutes. For the same period the second tube (*A*) was equilibrated with outside air. In every case a sample of gas was collected from the low pressure tube toward the end of the equilibration, and its later analysis accepted as giving the value of the initial oxygen and carbon dioxide percentages. The air which had passed through tube *A* was analyzed in several experiments and this value accepted for the rest as giving the initial oxygen and carbon dioxide percentages in the high pressure tube. It showed, after passing through the egg suspension, a slight diminution in oxygen and a slight increase in carbon dioxide.

At the conclusion of the equilibration the two tubes were closed

in such a manner that the contained gas was left at atmospheric pressure and at approximately 20° C. They were then placed side by side within a water bath, and rotated continually throughout the experiment, turning at the rate of about thirty times a minute. Under these conditions the eggs were always evenly distributed throughout the suspension, and kept in constant motion, the water was always nearly in equilibrium with the gas, and cleavage proceeded in a perfectly normal manner.

Running sea water was used in the water bath. Its temperature varied slightly from day to day. The lowest temperature recorded in any experiment was 18.2° C., the highest 20.2° C. The experiments continued in most cases for two hours; in a few cases for three hours. The first division occurs about one hour after fertilization at this temperature; subsequent divisions follow about every thirty minutes. At the end of the two-hour experiments the eggs were in the four and eight cell stage; at the end of the three-hour experiments they were in the sixteen and thirty-two cell stage. The material is not, therefore, unicellular throughout the whole experiment. The individual cells, however, in all of these early stages are all at the surface of the dividing egg in intimate relation with the oxygen supply in the water; there seems every reason to believe that the relationship under investigation will not be materially modified by this increase in number of cells, unaccompanied by any change in the mass of respiring tissue. We have reason to believe from the work of Gray (1925), that cleavage itself does not affect the rate of oxygen consumption, and that, after the first sharp rise following fertilization the consumption is practically constant during the first three hours of development. The unfertilized egg has so low a gaseous exchange that it has not proven practicable to follow its respiration by the present method.

At the end of the experiment samples of gas were secured from both tubes and analysed. The oxygen and carbon dioxide in the gas and in the sea water were then calculated for the beginning and for the end of the experiment. For this calculation the absorption coefficients for oxygen and carbon dioxide in sea water given in Tabulæ Biologicæ (Vol. 4, pp. 571–578) were used. The results of a typical experiment are as follows:

	Tube A.	Tube B.
Oxygen tensions during experiment.	155 to 142 mm. Hg.	61 to 49 mm. Hg.
Volume of tube	106.15 c.c.	105.39 c.c.
Volume of suspension	60 c.c.	60 c.c.
Gas Analysis at beginning:		
O_2	20.87%	8.22%
CO_2	.05%	.02%
N_2	79.08%	91.76%
Gas analysis at end (corrected for volume change):		
O_2	19.16%	6.59%
CO_2	.62%	.58%
N_2	79.08%	91.76%
Oxygen in air and water:		
At beginning	9.961 c.c.	3.863 c.c.
At end	9.147 c.c.	3.095 c.c.
Oxygen Consumption	.814 c.c.	.768 c.c.
Carbon dioxide in air and water:		
At beginning	.045 c.c.	.018 c.c.
At end	.558 c.c.	.517 c.c.
Carbon dioxide production	.513 c.c.	.499 c.c.
Volumes corrected to dry values at O° C. and 760 mm. Hg.		
Oxygen consumption	.741 c.c.	.699 c.c.
Carbon dioxide production	.467 c.c.	.454 c.c.
Respiratory quotient	.630	.649

Oxygen consumption at low pressure = 94.4% of that at atmospheric pressure.

Carbon dioxide production at low pressure = 97.3% of that at atmospheric pressure.

The results obtained in twenty experiments, carried out after the preliminary tests, are given in Table 2, and shown graphically in Fig. 1. The oxygen consumption is seen to be practically constant from an oxygen pressure of 228 mm. Hg. down to about 20 mm. Hg. Between 80 and 20 mm. there is a definite downward trend in the values, but at 20 mm. the consumption is still about 90 per cent. of that at atmospheric pressures. Below this point the consumption falls off sharply.

In Fig. 1 the experimental values are shown as rectangles. The height of this rectangle corresponds to 1 per cent. on the oxygen consumption scale; the length indicates the oxygen tension range in tube *B* during the course of the experiment. Each rectangle shows that over this range the oxygen consumption of the egg suspension in tube *B* was the indicated percentage of the consumption in tube *A*, run at atmospheric pressure. The absolute

TABLE II.

RESPIRATION OF FERTILIZED *Arbacia* EGGS AT DIFFERENT OXYGEN TENSIONS.

Experiment.	Oxygen Pressure in Tube B.	Respiration in Tube A.			Respiration in Tube B.			Ratio between O_2 Consumption in Tube B and that in Tube A.
		O_2 Cons.	CO_2 Prod.	R. Q.	O_2 Cons.	CO_2 Prod.	R. Q.	
	mm. Hg.	*c.c.	c.c.		c.c.	c.c.		
1	228.8–220.0	.423	.394	.931	.433	.294	.679	1.024
2	155.2–147.2	.473	.430	.909	.470	.386	.821	.994
3	152.2–144.6	.443	.329	.742	.447	.350	.783	1.009
4	142.0–135.7	.309	.257	.832	.315	.237	.753	1.019
5	123.2–112.4	.524	.436	.832	.533	.326	.611	1.017
6	116.8–104.3	.691	.443	.641	.733	.448	.611	1.061
7	85.5– 76.0	.650	.496	.763	.621	.403	.648	.955
8	70.6– 61.7	.572	.417	.729	.520	.412	.792	.909
9	66.6– 55.4	.665	.528	.794	.653	.493	.754	.982
10	61.2– 49.0	.741	.467	.630	.699	.454	.649	.944
11	44.6– 38.3	.390	.334	.856	.370	.339	.915	.949
12	36.8– 24.2	.818	.611	.768	.735	.535	.727	.899
13	30.0– 24.2	.406	.279	.688	.345	.217	.628	.850
14	23.9– 14.9	.592			.524			.885
15	23.7– 8.7	.856	.593	.697	.862	.558	.648	1.007
16	17.3– 10.1	.674	.444	.658	.419	.360	.859	.622
17	11.5– 6.3	.636	.543	.854	.367	.427	1.160	.577
18	7.9– 3.3	.565	.667	1.181	.268	.269	1.004	.457
19	7.1– .8	.746	.527	.706	.309	.488	1.582	.414
20	4.3– 1.7	.665	.445	.669	.151	.222	1.473	.227
		Average R. Q. .783			Average R. Q. (1–16) .725			

* Volume measured at 760 mm. Hg. and O° C.

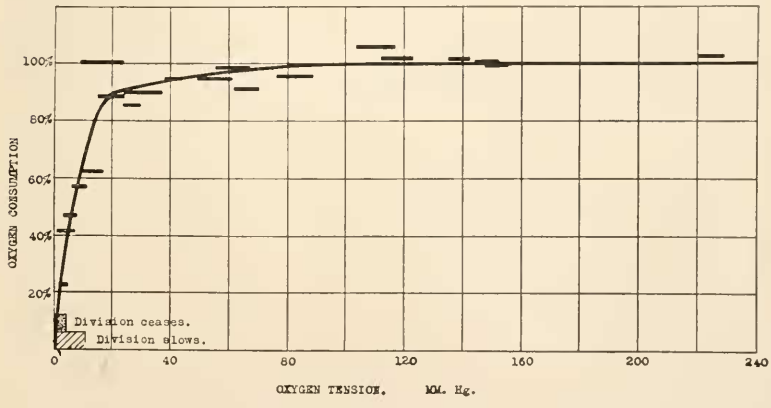

FIG. 1. Oxygen consumption of fertilized *Arbacia* eggs at different oxygen tensions. The range of tensions within which the division rate is affected is also graphically shown.

values vary considerably from experiment to experiment, but the graph of these percentages assumes a fairly regular and consistent form.

Correlated with the diminished oxygen intake at very low oxygen tensions retardation in development was observed in experiments 17–20. In all other experiments the eggs in the low pressure tube had developed as far as had those at atmospheric pressure. In every case 95–100 per cent. of the eggs developed. In experiment 17, continuing for two hours, a slight retardation in division rate was evident. Counts on 100 eggs from each suspension gave the following values:

	1-cell.	2-cell.	4-cell.	8-cell.
Tube *A* (High O_2)	4	6	57	33
Tube *B* (Low O_2)	5	14	74	7

In experiment 18 (2 hours) a more marked effect was observed. Counts on 100 eggs gave the following values:

	1-cell.	2-cell.	4-cell.	8-cell.	16-cell.
Tube *A* (High O_2)	2	0	52	40	6
Tube *B* (Low O_2)	54	39	7	0	0

In experiment 19 (3 hours) the eggs at atmospheric pressure were in the sixteen and thirty-two cell stage. In tube *B* about 80 per cent. had reached the four-cell stage, but none were found in later stages. In experiment 20 (2 hours) the eggs at atmospheric pressure were in the four and eight-cell stage. In tube *B* a careful search failed to reveal any cleavage whatsoever. It has long been known that in the complete absence of oxygen cleavage in these eggs is prevented. (See E. B. Harvey, 1926.) My own observations would suggest that a certain minimal concentration of oxygen is necessary for division, but the matter has not received a thorough study. The range of oxygen tensions within which development is either retarded or prevented is indicated graphically in Fig. 1. The values, taken from four experiments, are to be considered as approximations only. Taken in conjunction with the curve of oxygen consumption they show the great ability of

these eggs to carry out a normal development down to very low oxygen tensions.

It is of interest to note that in all four of these experiments in which retardation or inhibition of development occurred the respiratory quotient rose above unity; in experiments 19 and 20 the quotient reached the high values of 1.58 and 1.47. These figures suggest the presence of anaerobic respiratory processes at these low oxygen tensions. It is not possible to be certain concerning the matter, since, under these conditions of oxygen lack, acid metabolites may collect in the suspension and liberate carbon dioxide from the carbonates of the sea water.

In none of these experiments has the tension of carbon dioxide risen to such a point that it can have materially affected developmental rate. Haywood (1927) has shown that, in high concentration, carbon dioxide behaves as a narcotic and completely prevents cleavage when its tension rises above 230 mm. Hg. Below this value cleavage occurs at a rate slower than normal. The threshold tension for this carbon dioxide effect to appear was not determined, but it seems evident that at very much lower concentrations the retardation of development must become negligible. The highest carbon dioxide value observed in the present study was at the end of experiment 18, when the partial pressure reached 7 mm. Hg in tube *B*. The retardation of development observed at low oxygen tensions must therefore be caused by oxygen lack rather than by a narcotic effect of the carbon dioxide produced. Haywood also reports experiments on the influence of low oxygen tension upon developmental rate which agree with my own findings in showing practically no influence down to quite low values.

In most experiments carried out below an oxygen tension of 50 mm. Hg there was observed, at the end of the experiment, a liberation of pigment in the suspension in the low pressure tube which became more and more marked as the oxygen tension was lowered. This liberation of pigment apparently arose from the cytolysis of a certain number of cells. The actual percentage of eggs thus destroyed was not determined, but must have been small, since at the end of the experiment the volume of the eggs after sedimentation was not appreciably diminished. The downward

trend in the oxygen consumption values below 80 mm. Hg may be in part due to this destruction of a small number of the eggs, although we know, from the work of Warburg (1914) that respiratory exchanges may continue for some hours even in completely fragmented sea-urchin eggs, at a level not far below that found when the cells are intact.

The ability of both protozoa and sea-urchin eggs to carry on a normal respiratory exchange down to very low oxygen tensions points very definitely to the normal presence, within the cells, of a considerable oxygen tension. Oxygen is present in such amount that it does not limit the metabolism, whose rate is determined by other than oxidative reactions.

Summary.

By standard methods of gas analysis the respiratory exchanges of *Paramecium* and of fertilized *Arbacia* eggs have been studied. The respiratory rate in both materials is found to be practically constant over a wide range of oxygen tensions, thus confirming older work done by other methods.

In the fertilized *Arbacia* egg the oxygen consumption is practically constant between 228 and 20 mm. Hg partial pressure of oxygen. Between 80 and 20 mm. Hg there appears to be a slight diminution in oxygen intake, but at 20 mm. Hg the consumption is still about 90 per cent. of that at atmospheric pressure. Below 20 mm. Hg the consumption is sharply reduced.

The cleavage of *Arbacia* eggs proceeds at a normal rate down to very low oxygen tensions. No retardation in development has been observed above 11 mm. Hg. Below this value the rate becomes slower and cleavage ceases entirely below 4 mm. Hg.

BIBLIOGRAPHY.

Alsterberg, G.

'26 Die Winklersche Bestimmungsmethode für in Wasser gelösten elementaren Sauerstoff. Biochem. Zeits., 170, 30–75.

Amberson, W. R., Mayerson, H. S., and Scott, W. J.

'24 The Influence of Oxygen Tension upon Metabolic Rate in Invertebrates. Journ. Gen. Physiol., 7: 171–176.

Burrows, M. T.

'24 Relation of Oxygen to the Growth of Tissue Cells. Amer. Jour. Physiol., 68: 110.

Gray, J.

'25 The Mechanism of Cell Division. Oxygen Consumption during Cleavage. Proc. Camb. Phil. Soc., 1: 225–236.

Harvey, E. B.

'27 The Effect of Lack of Oxygen on Sea Urchin Eggs. Biol. Bull., 53: 147–160.

Harvey, E. N.

'25 The Total Luminous Efficiency of Luminous Bacteria. Jour. Gen. Physiol., 8: 89–108.

Haywood, C.

'27 Carbon Dioxide as a Narcotic Agent. Biol. Bull., 53: 450–464.

Heilbrunn, L. V.

'15 The Measurement of Oxidation in the Sea-Urchin Egg. Science, N. S., 42: 615–616.

Henze, M.

'10 Über den Einfluss des Sauerstoffdrucks auf den Gaswechsel einiger Meerestiere. Biochem. Zeits., 26: 255–278.

Lund, E. J.

'18 Relation of Oxygen Concentration and the Rate of Intracellular Oxidation in *Paramecium Caudatum*. Biol. Bull., 45: 351–364.

Novy, F. G., and Soule, M. H.

'25 Respiration of the Tubercle Bacillus. Jour. Inf. Dis., 36: 168–232.

Pütter, A.

'24 Die Atmung der Planktonbakterien. Arch. ges. Physiol., 204, 94–126.

Stephenson, M., and Whetham, M.

'24 The Effect of Oxygen Supply on the Metabolism of Bacillus Coli Communis. Biochem. Jour., 18: 498–506.

Warburg, O.

'08 Beobactungen ñber die Oxydationsprozesse in Seeigelei. Zeits. Physiol. Chem., 57: 1–16.

'14 Zellstruktur und Oxydationsgeschwindigkeit nach Versuchen am Seeigelei. Arch. ges. Physiol., 158: 189–208.

'14 Über die Rolle des Eisens in der Atmung des Seeigeleis. Zeit. Physiol. Chem., 92: 231–256.